role of the dynamin GTPase activity in controlling fusion pore expansion and post-fusion granule membrane topology was investigated using the powerful membrane curvature-imaging technique of polarized TIRF microscopy and amperometry. A dynamin-1 mutant with increased GTPase activity resulted in faster fusion pore widening and flattening of the granule membrane after exocytosis; dynamin-1 mutants with decreased activity slowed fusion pore widening by stabilizing post-fusion granule membrane curvature. The experiments indicate that the GTPase activity of dynamin functions as a timer determining the rapidity of fusion pore expansion from 10's of milliseconds to seconds after fusion, in addition to its role in endocytosis. These findings expand the membrane-sculpting repertoire of dynamin to include the regulation of immediate post-fusion events in exocytosis that control the rate of release of soluble granule contents.

159-Plat
Inhibition of F-Actin Cycling in Rbl Mast Cells Prevents Endosome Acidification but not Internalization of Antigen-Crosslinked IgE Receptor Complexes
Amit Singhai, David Holowka, Barbara Baird.
Cornell University, Ithaca, NY, USA.
Previous studies have implicated roles for the actin cytoseleton in endocytosis via clathrin-coated pits, as well as in antigen-stimulated endocytosis of immunoglobulin-E (IgE) receptors (Ra et al., Eur. J. Immunol. 19:1771, 1989). To monitor endocytosis in real time, we use a fluorescence assay that reports pH-dependent quenching of FITC-IgE. We find that micromolar concentrations of either cytochalasin D or latrunculin A, inhibitors of actin polymerization, as well as jasplakinolide, a stabilizer of F-actin polymerization, all prevent this antigen-stimulated FITC quenching due to endosomal acidification. Under these conditions, crosslinked IgE receptors appear localized in endosomes by confocal imaging, despite the lack of acid-dependent FITC quenching. A quantitative flow cytometry assay for surface-available IgE shows that greater than 60% of these IgE receptor complexes are internalized in response to antigen crosslinking in the presence or absence of these inhibitors of actin polymerization cycling. After 20 min at 37oC with antigen, we observe partial co-localization of IgE receptor-containing endosomes with a lysosomal marker, indicating normal endosomal trafficking of these complexes in the presence or absence of these agents. Addition of latrunculin A or cytochalasin D after antigen stimulated endocytosis causes rapid, partial reversal of endosomal acidification monitored by FITC-IgE, suggesting dynamic regulation of this process by the actin cytoskeleton. In contrast, secretory lysosomes labeled with FITC-dextran by fluid-phase pinocytosis maintain normal acidification in cells treated with these agents. In ongoing experiments we are investigating the molecular mechanism for cytoskeletal regulation of endosomal acidification.

160-Plat
Understanding the Fundamentals of Platelet Granular Storage and Release at Single Cell Level
Secil Koseoglu, Christy L. Haynes.
University of Minnesota, Minneapolis, MN, USA.
Platelets are traditionally recognized as critical cells in hemostasis and thrombosis but have also recently been identified to play a significant role in many diseases, including bacterial infections, cancer, and allergic asthma. Platelets store and release important messenger molecules via exocytosis from two populations of granules (α- granules with adhesive protein species and δ- granules with small molecule/ion species). However, very little known about how chemical messengers are stored in these granules and the driving forces for secretion into the blood stream, thus limiting the development of new therapeutic approaches to manage the role of platelets in many physiological events. Carbon-fiber microelectrode amperometry (CFMA) enables both quantal and kinetic analysis of the exocytotic event of single cell with sub-ms time resolution but is limited to measuring electroactive species from δ-granules. Localized surface plasmon resonance (LSPR) spectroscopy is a complementary tool that offers an outstanding sensing ability by measuring the extremely small changes in the sensing media. This work aims to overcome the gap in fundamental knowledge about platelet granular storage and secretion by detecting serotonin secretion from δ- granules using CFMA and Platelet factor 4 (PF4) secretion from α- granules using LSPR. These measurements are performed while exposing platelets to different stimulants, including ionomycin, adenosine diphosphate (ADP), and thrombin. Significant differences are seen in both α- and δ-granule secretion with varied stimulant exposure, and these combined measurements yield biophysical insight into the mechanism behind these secretory behaviors.

161-Plat
Low Physiological Level of Stimulation Elicits Exocytosis, not by Ca^{2+} Influx, but by Suppression of Ca^{2+} Syntillas in Mouse Adrenal Chromaffin Cells
Jason J. Lefkowitz, Kevin E. Fogarty, Kailai Duan, John V. Walsh, Jr, Valerie De Crescenzo.
University of Massachusetts Medical School, Worcester, MA, USA.
Catecholamine and neuropeptide exocytosis from adrenal chromaffin cells (ACCs) is controlled by sympathetic autonomic discharge. It has been thought that sympathetic discharge accomplishes this task exclusively by regulating Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs), which serves as a direct trigger for exocytosis. But our studies on spontaneous exocytosis in ACCs revealed the presence of Ca^{2+} syntillas, focal cytosolic transients mediated by ryanodine receptors (RYRs), and these had the surprising effect of *inhibiting* spontaneous exocytosis (Lefkowitz et al., 2009).
Here we examine the role of syntillas under physiologic stimulation in ACCs using simulated action potentials (sAPs) designed to mimic native input at a frequency associated with basal sympathetic tone: 0.5 Hz. Stimulation at this frequency induces a general increase in the frequency and size of amperometric events comparable to that observed when syntillas were suppressed under spontaneous release conditions. Unexpectedly, we found that sAPs delivered at 0.5 Hz completely abolished Ca^{2+} syntillas within two minutes. Ca^{2+} arising from VGCCs was not enough to elevate the global $[Ca^{2+}]_i$ as measured with fura-2. Hence, it appears that *inhibition* of syntillas by action potentials in ACCs is responsible for the increase in exocytosis at this level of stimulation.
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Lefkowitz, J.J., K.E. Fogarty, L.M. Lifshitz, K.D. Bellve, R.A. Tuft, R. ZhuGe, J.V. Walsh, Jr., and V. De Crescenzo. 2009. Suppression of Ca^{2+} syntillas increases spontaneous exocytosis in mouse adrenal chromaffin cells. *J Gen Physiol* 134(4):267-280.

162-Plat
Complexin Facilitates Coupling of Secretory Vesicles and Voltage-Gated Calcium Channels
Joyce G. Rohan[1], Sonia Ming-yi Lin[1], Jung Hwa Cho[1], Haijiang Cai[2], Kerstin Reim[3], Nils Brose[3], Chien-Ping Ko[1], Robert H. Chow[1].
[1]University of Southern California, Los Angeles, CA, USA, [2]California Institute of Technology, Pasadena, CA, USA, [3]Max Planck Institute for Experimental Medicine, Goettingen, Germany.
The SNARE complex mediates fusion of vesicle and plasma membranes in exocytosis. Complexin (Cplx) binds with high affinity and 1:1 stoichiometry to the SNARE complex and is believed to regulate SNARE function. However, how Cplx functions is still controversial. Using mouse neuromuscular junctions (NMJs) and adrenal chromaffin cells, we tested the hypothesis that Cplx, in addition to enlarging the pool of primed vesicles, enhances exocytosis and neurotransmission by increasing the coupling between vesicles and voltage-gated calcium channels (VGCC). Only Cplx 1 is expressed at adult mouse NMJs. Cplx 1 knockout NMJs displayed not only a diminished endplate potential (EPP) amplitude, but also a loss of synchronicity ("jitter"). Furthermore, whereas in response to high-frequency stimulation, wildtype NMJs displayed marked depression of EPP size upon successive stimulations, knockout NMJs showed facilitation. These observations suggest that in Cplx knockout NMJs, vesicles and calcium channels are uncoupled. To further test this hypothesis, we recorded capacitance measurements from mouse adrenal chromaffin cells. We used short depolarizations to elicit fusion of vesicles close to the calcium channels (IRP) and longer depolarizations to elicit fusion of the remaining primed vesicles (RRP). Cplx 2 KO chromaffin cells have a significantly smaller IRP/RRP ratio, and we can rescue the phenotype by heterologous expression of complexin in KO cells. Our work in two separate model systems supports a role for complexin in coupling vesicles to VGCC.

163-Plat
Complexin and Synaptotagmin Genetically Interact in vivo Controlling the Rates of Spontaneous and Nerve-Evoked Vesicle Fusion
Ramon A. Jorquera, Sarah Huntwork, J. Troy Littleton.
MIT, Cambridge, MA, USA.
Presynaptic vesicle fusion is a highly regulated process that requires Synaptotagmin (SYT) and Complexin (CPX) proteins. Current data suggest that SNARE binding by the two proteins may be co-regulating the last step of release. However, their precise roles in synaptic function are still under debate. Here, we present a thorough analysis of synaptic transmission of

null-mutants and over-expression transgenic animals for CPX and SYT at the Drosophila NMJ. Genetic interaction and kinetics analysis reveals that CPX and SYT expression modulate the rates of spontaneous and synchronous/asynchronous release. Additionally, SYT and CPX levels alter vesicle delivery during tetanic stimulation. Our finding indicate that different levels of SYT and CPX have computational consequences at nerve terminal that modulate basal noise, output gain, speed of transmission and the short-term plasticity.

PLATFORM N: Regulatory Networks & Systems Biology

164-Plat
Construct and Simulate Virtual Cell with Minimum Genome at the Nanoscale
Yu Xu[1], Ruth Nussionv[2], **Buyong Ma**[2].
[1]Institute of Chinese Minority Traditional Medicine,Minzu University of China., Beijing, China, [2]SAIC-Frederick, NCI-Frederick, NIH, Frederick, MD, USA.
Understanding the design principles of living systems at the nanoscale presents both challenge and promise for controlling cancer cell. We constructed and simulated a virtual cell model containing 206 proteins in the minimal bacterial genome. All the molecules are treated as nano-scale spheres. The short-range attraction interactions between those macromolecules are described by the Lennard-Jones potential, and electrostatic interactions by the point charge model. The motion of the protein particles are simulated with Langevin Dynamics. 8 copies of the Green Fluorescent Proteins are also added as benchmark to compare the particle diffusion characteristics of the model with experiment results in vivo. With three parameters (well-depth of Lennard-Jones potential, point charges, and viscosity of the cytoplasm), we reproduced the experimental diffusing constant for GFP. We found that in virtual cell simulation, the selection of protein and their concentration are very important to represent cellular life. The spatial and temporal distributions of cytoplasmic proteins are not homogenous. The hierarchical protein clusters may provide spatial pathways for protein-protein interaction networks. This project has been funded by the NCI, contract number HHSN261200800001E.

165-Plat
Toward a Whole Cell Model of Mycoplasma Genitalium
Jonathan R. Karr, Jayodita C. Sanghvi, Jared M. Jacobs, Markus W. Covert.
Stanford University, Stanford, CA, USA.
A central challenge in biology is to understand how cellular life emerges from individual biochemical interactions. To address this challenge we have developed a novel computational framework which facilitates the integration of multiple disparate biochemical networks and data into a single unified model. Using this framework we have developed a detailed computational model of the complete life cycle of the smallest known freely-living organism, Mycoplasma genitalium. The model describes the life cycle of a single cell including DNA, RNA, and protein synthesis, metabolism, and cell division. The model accounts for the specific function of every annotated gene product, and simulates the dynamics of every molecular species. We have validated the model using several publically available experimental datasets. Currently we are using the model to gain insight into the control and regulation of cellular growth by exploring the effects of genomic and environmental perturbations on cellular behavior. In addition, we are developing an open-source, open-access web-based platform to facilitate broad application of our model.

166-Plat
Nonspecific Binding Limits the Number of Proteins in a Cell and Shapes their Interaction Networks
Margaret E. Johnson, Gerhard Hummer.
National Institutes of Health, Bethesda, MD, USA.
Multicellular organisms, from the simple worm to humans, have roughly the same number (about 20,000) of protein encoding genes. We find that the need to prevent disease-causing nonspecific interactions between proteins provides a simple physical reason why organism complexity is not reflected in the number of distinct proteins. Through computational evolution of the amino-acid sequences of protein binding interfaces with an empirical energy function, we quantify the degree of mis-binding as a function of the number of distinct proteins. We show that the achievable energy gap favoring specific over nonspecific binding decreases with protein number in a power-law fashion. We demonstrate how the scaling of this power law depends on the size of the binding interfaces and the topology of the protein interaction network. We predict the limits these binding requirements place on the number of different proteins that can function effectively in a given cellular compartment by calculating the fraction of proteins involved in nonspecific complexes as a function of increasing protein number and decreasing energy gap. Remarkably, the optimization of interfaces favors networks in which a few proteins have many partners, and most proteins have few partners, consistent with a scale-free network topology. We conclude that nonspecific binding adds to the evolutionary pressure to develop scale-free protein-protein interaction networks.

167-Plat
Exact Identification of Topologically Essential Interactions in the Networks Derived from Perturbation Experiments
Maxim N. Artyomov[1], Ana Paula Leite[1,2], Aviv Regev[1,2].
[1]Broad Institute, Cambridge, MA, USA, [2]MIT, Cambridge, MA, USA.
Perturbation screens (e.g. by gene knock-downs) are one of the most promising tools available for discovering the structure of complex biological networks. Considerable obstacle in understanding the results of such screens stems from inability to distinguish the indirect effects of a perturbation from the direct effects. Thus, networks derived from typical perturbation screen are significantly more complex than true underlying networks. The problem of identifying minimal core network topology consistent with results of perturbation screen (i.e. discriminating between direct and indirect effects of a perturbation) has been accurately formulated previously but despite the attempts to solve it, only approximate methods with severe limitations have been developed. Here, we report a novel approach that is based on the theory of self-avoiding random walks which allows one to find an exact solution to the problem: given experimentally derived network one can identify core network(s) consistent with original network (note that for sufficiently complex network, more than one core network is possible). By introducing novel matrix representation of the network topology we reduce the problem of identifying core underlying networks to the counting of self-avoiding random walks in the original network, thus allowing exact solution for any input network topology. We describe application of our approach to synthetic data obtained by simulating artificially constructed networks, as well as to the results of real perturbation screens performed in yeast and mammalian systems.

168-Plat
Detection of Predictive Dynamics of Glucocorticoid Receptors in Xenopus Laevis Embryonic Tissues
YongTae Kim[1], Sagar D. Joshi[2], Philip R. LeDuc[1], Lance A. Davidson[2], William C. Messner[1].
[1]Carnegie Mellon University, Pittsburgh, PA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA.
The regulatory interactions in embryonic development that is guided by a complex set of spatially and temporally integrated stimuli result in the emergence of highly functional units. Since a better understanding of spatiotemporal responses provides a great insight to fundamental emergent patterns that may evolve from complex systems such as embryonic development, it is important to examine a generalized response to frequency-modulated stimulation from a system comprised of hundreds of cells where each cell has millions of interacting molecules. We present the dynamic responses of vertebrate embryonic tissues to time-varying localized chemical stimulation through a closed-loop microfluidic control that enabled localized spatiotemporal regulation of steroid hormone dexamethasone (DEX) in Animal Cap (AC) tissues isolated from gastrulating Xenopus embryos. We investigated dynamic cell-scale responses to precisely controlled stimulation by tracking the activity of a GFP-tagged Glucocorticoid Receptor (GR). Interestingly, the overall response had a predictive first-order behavior to periodic stimulation environments. We modeled these dynamic responses using first order differential equation with two different time derivatives: moving into and out of the nucleus. There was a good agreement between the predicted responses and the experimental results. Our approach provided a methodology for manipulating these biochemical inputs to examine and model the collective behavior of many biochemical reactions. The intricate cellular signaling and transport machinery responses were similar to behaviors in other complex systems, suggesting that even within a highly integrated and robust embryonic tissue, the overall system that we had studied converged toward a predictive first-order response. We believe that our results may suggest in the future that nanoscale molecular interactions in this developmental system results in highly regulated behavior, which we are able to determine through our feedback microfluidic control approaches in developmental biology.